# METHANE AND NITROUS OXIDE FLUXES IN AN ACID OXISOL IN WESTERN PUERTO RICO: EFFECTS OF TILLAGE, LIMING AND FERTILIZATION

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**Summary**—Changes in land use and management of tropical systems are considered to be major factors in the recent upsurge in increases in atmospheric nitrous oxide ($N_2O$) and methane ($CH_4$). Studies were initiated in western Puerto Rico grasslands to determine the effect of plowing, or liming and fertilizing an acid Oxisol on the soil–atmosphere exchanges of $N_2O$ and $CH_4$. Weekly field flux measurements and field manipulation and laboratory studies were conducted over 22 months during 1993–1995. We found that $N_2O$ emissions from an Oxisol acidified to pH 4 were generally lower than from pH 6 Oxisol soils that were used as reference controls. Plowing the grasslands did not change mean $N_2O$ emission rates from either pH soil. Liming the acidified Oxisol to pH 6 tended to increase $N_2O$ emissions to the rates from the undisturbed grassland. Fertilizing the acidified grassland increased $N_2O$ emissions but much less than when these soils were both limed and fertilized. Short-term field studies employing nitrification inhibitors in which we measured nitric oxide (NO) and $N_2O$ emissions, demonstrated that nitrification rates generally control $N_2O$ emissions; thus these were lower in unlimed soil. It is likely that NO was produced through the chemical decomposition of nitrite, which in turn, was a product of biological nitrification. Soil consumption of atmospheric $CH_4$ in the acidified Oxisol was about one-fourth of that in the pH 6 reference soil. Liming did not restore $CH_4$ consumption in the acid soil to rates comparable to those in the reference Oxisol. We conducted a laboratory induction study to determine if incubation of these limed or unlimed acidified soils with high concentrations of $CH_4$ could induce methanotrophic activity. Comparable uptake rates to the control soils were not induced by these incubations. These studies illustrate that management of soil can considerably affect the soil–atmosphere exchange of such trace gases as $N_2O$ and $CH_4$ which can affect global atmospheric properties. Published by Elsevier Science Ltd

## INTRODUCTION

Methane and nitrous oxide are long-lived atmospheric trace gases that are collectively responsible for about 25% of anticipated global warming. The soil production and consumption of these gases comprises more than 50% of the atmospheric budget (Cole *et al.*, 1996). Since soil microorganisms play a large role in the global budgets of these gases it is important to understand how their soil–atmosphere exchange is regulated in different ecosystems. Tropical soils are considered to play an important role in the atmospheric budgets of both gases (Matson and Vitousek, 1987; Keller and Matson, 1994; Potter *et al.*, 1996a,b). Even so, the role that soil management and chemicals factors, such as soil pH, have on the production and consumption of these gases is not fully understood.

Although the microorganisms which take part in soil N transformations typically function optimally at near neutral pH conditions (Paul and Clark,

1996) nitrification can proceed rapidly at low pH. In moist tropical forest soils, significant net nitrification rates at pH 5 have been observed (Neill *et al.*, 1995). In these systems, soils converted from forest to pasture increased in pH but had lower mean annual net mineralization and net nitrification rates than the original forest soils. Tropical agricultural soils are frequently highly weathered and have a low pH. In these agricultural soils, N transformations such as nitrification are typically slow. Management practices such as liming, which strive to make these soils more amenable to growing crops, tend also to increase rates of N turnover, nitrification and denitrification and influence the ratio of $N_2O$-to-$N_2$ production during denitrification (Alexander, 1965). Moist, temperate grassland soils can respond similarly to liming. Utilizing experimental grassland sites, that were established in 1856 at the Rothamsted Experimental Station in England, that had been limed from pH 3.9 to 5.9 and 7.6 since 1903, Yamulki *et al.* (1997) found that mean $N_2O$ emissions increased about 4.5-fold from pH 7.6 soils compared to pH 3.9 soils.

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Generally, as pH declines, the rates of organic N mineralization, ammonification, nitrification and denitrification also decline (Alexander, 1965; Broadbent and Clark, 1965; Jackson, 1967). Autotrophic nitrification rates are typically very low at pH 4 and below (Jackson, 1967). Alexander (1965) noted that it is common knowledge that nitrification proceeds slowly in acid agricultural soils and is hastened by liming. Nitrification does, however, proceed in soil below the pH limits observed in pure culture, but a pH limitation of about 4 is typical (Schmidt, 1982). Observance of heterotrophic nitrification has been confined primarily to acid forest soils (Paul and Clark, 1996).

Although denitrification rates are typically much lower in acid soils, pH < 5, and peak at pH above 6 (Broadbent and Clark, 1965), denitrification activity at pH 3.6 has been observed (Firestone, 1982). Even in acid peat soils denitrification rates are typically low (Payne, 1981). At pH below 5, nitrous acid decomposes to promote significant chemodenitrification in very specific situations (Broadbent and Clark, 1965).

In studies with acid spruce forest soils, Priha and Smolander (1995) found that N addition to a pH 4.1 soil produced little denitrification. When they added ammonium to soil from the same location that was limed to pH 5 a large amount of $N_2O$ through denitrification was produced, but not through nitrification. In an acid soil beech forest, pH 4.5, Brumme and Beese (1992) found that liming to pH 6.5 decreased $N_2O$ emissions by 74%. They suggested that the decrease in $N_2O$ emission was due to the change in the $N_2O$-to-$N_2$ ratio and not to a change in denitrification rate. This decrease in $N_2O$-to-$N_2$ of the denitrification product ratio with increasing pH is a typical observation (Firestone, 1982; Klemedtsson et al. 1997). Firestone (1982) noted that the pH shift may have little effect on total $N_2 + N_2O$ produced but that the relative effect of decreased pH on $N_2O$ reductase may regulate the $N_2O$-to-$N_2$ ratios.

Methane oxidation by soil microorganisms generally appears to be less limited by soil pH than nitrification (Knowles, 1993). Methane consumption in peat soils at pH below 4 has been observed (Moore and Knowles, 1990). Sitaula et al. (1995) found that adding pH 3 irrigation water to an acidic Scots pine forest soil increased soil $CH_4$ consumption relative to irrigation with pH 5.5 water. They concluded that these pH effects either reflected a difference in the communities of methanotrophs or a change in the amounts of activity. Klemedtsson and Klemedtsson (1997) found that liming a Swedish forest soil from pH 4 to 5.3 had little effect on $CH_4$ uptake but addition of 35 kg N $ha^{-1}$ $yr^{-1}$ decreased $CH_4$ uptake by about 60%.

In most systems the addition of nitrogen fertilizers decreases $CH_4$ consumption in the soil (Bedard and Knowles, 1989; Steudler et al., 1989; Mosier et al., 1991). This added N decrease has generally been attributed to a competitive effect of ammonium with nitrifiers that also oxidize $CH_4$ (Steudler et al., 1989; Knowles, 1993) and to the effect of increased N cycling following N additions (Mosier et al., 1991, 1996). Sitaula et al. (1995) observed no interaction between soil acidification and N deposition on $CH_4$ uptake and concluded that nitrifiers were of minor importance to $CH_4$ oxidation in their forest soils. Crill et al. (1994) observed that the fertilizer N effect in a drained peatland soil was not simply dependent upon ammonium concentration but also possibly to the effect of added salts, $K^+$ and $Cl^-$. Addition of salts of KCl, NaCl, $KHCO_3$ or $K_2SO_4$ decreased $CH_4$ oxidation by about the same amount as adding $KNO_3$ (Nesbit and Breitenbeck, 1992; Adamsen and King, 1993; Bronson and Mosier, 1993).

Cultivation of a tropical forest soil in Panama generally decreased soil $CH_4$ uptake (Keller et al., 1990). Fertilization of cultivated soils where $CH_4$ consumption rates were already very low did not further decrease $CH_4$ consumption in irrigated crops in Colorado (Bronson and Mosier, 1993), but Hansen et al. (1993) and Willison et al. (1994) observed further declines in $CH_4$ uptake in cultivated soils with N additions. Mosier and Delgado (1997) found a variety of responses to N additions to three different grassland soils in western Puerto Rico. Fertilizer addition had a small negative effect on $CH_4$ uptake in a Vertisol, tended to enhance $CH_4$ uptake in an Ultisol and decreased $CH_4$ uptake in an Oxisol.

We have determined the effect of tillage, cropping and management on the soil–atmosphere exchange of $CH_4$ and $N_2O$ on Oxisol (pH 6) and acidified Oxisol (pH 4) soils.

## MATERIALS AND METHODS

### Control Oxisol site description

In late July 1992, a field site was established in a grassland on the USDA/ARS research station located near Isabela in the northwest corner of Puerto Rico (18°28′40″N latitude and 67°2′30″W longitude). At this site the 40-yr average precipitation is 1650 mm and the mean annual temperature is 24°C. The soil within the grassland is an Oxisol classified as a Coto clay (very fine, kalonitic, isohyperthermic Typic Hapludox) that had developed from limestone. The soil had a bulk density of 1.36 g $cm^{-3}$ in the top 10 cm of soil, a pH of 6.4, total C and N contents of 2.1 and 0.24% and had a gravimetric water content of 10.7, 21.5 and 32.2% at soil water filled pore space of 30, 60 and 90%. The dominant grass at the site was Guinea grass (Panicum maximum). The cultivation and use record of the site before it became an agricultural exper-

imental station was not known, but the site had not been plowed during the past 25 y and was left undisturbed. Within this pasture 12, 7 × 6 m plots were established within a 36 × 14 m area with six fertilizer treatment (O-F) and six unfertilized control plots (O-C) assigned randomly. At 3 month intervals, beginning 27 July 1992, fertilized plots received a surface application of 500 kg ha$^{-1}$ of 15–5–10 mixed fertilizer which contained 60 kg ha$^{-1}$ of ammonium sulfate-N and 15 kg ha$^{-1}$ of ammonium phosphate-N with each fertilization (Mosier and Delgado, 1997).

### Acidified Oxisol plots description

About 100 m from the Oxisol plots was an experimental plot that had not been used since 1991 and had reverted to a grassland having similar plant community and composition to the Oxisol plots. The soil within this 1 ha site (initial pH about 6) had been acidified by adding ammonium sulfate to accommodate field trials of acid-tolerant crops in 1987. Sorghum, fertilized with 133 kg N ha$^{-1}$ of ammonium sulfate, was grown on the plots in 1990. In October 1993 we measured soil pH (0–10 cm) on several transect across the plots and found that a relatively uniform pH across the plots of 3.9 to 4.5 was still present.

### Field experiments within the Oxisol (O) and acidified Oxisol (AO) plots

*Experiment 1: Tillage and cropping* About 5 m from the Oxisol sites (O-C and O-F plots), across the north boundary, a 5-m wide × 36-m long strip of grassland was plowed (15–20 cm deep), then disked to break up the sod and then rototilled (about 10 cm deep) to smooth the soil surface (site O-P). A second area of the same size was plowed and smoothed within the acidified Oxisol area (AO-P). Within both plowed areas six replicate plots were established with each plot containing one chamber anchor (for fixed gas sampling locations) and an additional six plots each containing one chamber anchors were established a few meter from the anchors in the plowed area of the AO plots to establish control plots within the acidified Oxisol (AO-C). A third flux measurement location was established within a sorghum breeding experimental plot located about 100 m from the Oxisol plot. The pH in the 15–30-cm plow layer of the sorghum field averaged 5.8 (5.2–6.1). This Oxisol field had been used as an experimental site for many years. After plowing and disking, sufficient fertilizer, 15–5–10, to provide 100 kg N ha$^{-1}$ was surface applied and the field was disked to incorporate the fertilizer. The field was then seeded with sorghum (*Sorghum bicolor* L.) on 5 October 1993. Immediately after seeding, chamber anchors were installed, three within sorghum rows and three between rows. Gas flux measurements in the sorghum field were made

weekly between 5 October 1993 and 8 March 1994 when the sorghum field was tilled following harvest. The comparison between the AO-C, AO-P, O-C, O-P, O-F and sorghum plots were made during this period. Gas flux measurements were made in the O-C and AO-C sites from 2 November 1993 until 25 October 1994.

*Experiment 2: Initial liming study* The effect of liming, to increase soil pH, on trace gas exchange was tested. Within the AO site a 36 × 8 m strip was plowed, disked and then divided into 12, 4 × 6-m plots. Six plots were randomly selected for liming with 1 kg m$^{-2}$ of commercial powdered lime. The lime was spread as uniformly as possible by hand then all the plots were rototilled (10–20 cm deep) to incorporate the lime and smooth the plot surface. Chamber anchors were installed and gas flux measurements were made 2 d after establishment then weekly from 5 July 1994 until 25 October 1994.

*Experiment 3: Liming and fertilization of the acidified Oxisol* We initiated a second, more detailed liming plus fertilization study in the AO site. In the center of the AO site an area 16 × 36 m was plowed (15–30 cm deep), disked and rototilled. Six replicate 4 × 6-m plots were randomly assigned to each of the following four treatments: control (AO-C), lime (AO-L), fertilizer (AO-F), or lime + fertilizer (AO-F-L). Lime was applied at the rate of 1 kg m$^{-2}$ and fertilizer, as described for the Oxisol site (75 kg N ha$^{-1}$) was broadcast. The plots were rototilled (about 20 cm) to incorporate the lime and fertilizer amendments. No further liming was done but the fertilized plots were fertilized at 3-month intervals with surface applications of the same rate of mixed fertilizer as used on the O-F plots. Chamber anchors were installed within each plot and gas flux measurements were made 2 h, 2 d, and 7 d after liming and fertilizing then continued weekly during the period from 25 October 1994 to 22 August 1995.

*Experiment 4: Effect of nitrite fertilization, nitrification inhibitors and ammonium fertilization on NO and N$_2$O emissions* Within the unfertilized limed (AO-L) and control plots (AO-C) of the AO site we also conducted a short-term experiment to determine the effects of liming, fertilization and nitrification inhibitors on NO and N$_2$O emissions. We installed five additional chamber anchors in the third week of April, 1995 within 4 limed and 4 unlimed, unfertilized plots. On 1 May 1995 we applied solutions (200 ml, 6.4 mm of water) containing water only, and water plus the equivalent of 0.75 kg N ha$^{-1}$ of NaNO$_2$, and fluxes of NO and N$_2$O were measured 0.5 and 4 h after nitrite application. The next day another experiment was started where the equivalent of 75 kg N ha$^{-1}$ of ammonium sulfate was added in 6.4 mm of water onto the microplots within each of four limed or unlimed plots. With the ammonium sulfate treatments we

applied in solution 7.5 kg ha$^{-1}$ dicyandiamide (DCD) or acetylene (about 30 min before making flux measurements) or nothing additional. Acetylene was applied by placing a N$_2$O/CH$_4$ flux chamber over a plot and injecting 60 ml of C$_2$H$_2$, generated from calcium carbide, into the chamber headspace. The chamber was sealed and left in place for 30 min to allow the C$_2$H$_2$ to diffuse into the soil, the chamber was removed and allowed to aerate for 5 min (Neff *et al.*, 1995). Fluxes of NO and N$_2$O were measured 0.5, 4 and 24 h after fertilizer addition. Nitrous oxide emissions were measured 8 d later from these plots.

*Gas flux measurements* Chamber anchors to establish each gas sampling location were installed in July 1992 within each O-C and O-F plot and at the times designated in each of the other experiments. The permanently placed anchors were made from 20.3 cm dia PVC pipe which was driven 8 cm into the soil (Mosier *et al.*, 1991, 1996). The inside top 1 cm of the anchor, which was above the soil surface, had been milled to allow fitting a chamber into the anchor. Each week the soil–atmosphere exchange of CH$_4$ and N$_2$O within each plot was measured by fitting a 7.5 cm high, closed, vented chamber (Hutchinson and Mosier, 1981) onto the anchor. We provided an additional seal around the anchor-chamber joint with a 3-cm wide rubber ring cut from a truck tire inner tube. Gas samples from inside the chamber were taken with 35 ml polypropylene syringes fitted with nylon stopcocks, 0, 15 and 30 min after installing the chambers. Gas samples were taken to the USDA/ARS laboratory in Mayaguez and analyzed for N$_2$O and CH$_4$ concentration by gas chromatography (Mosier *et al.*, 1991; Mosier and Delgado, 1997). Gas fluxes were measured weekly during the periods prescribed for each part of the studies described below.

Fluxes of nitric oxide (NO) were measured on two different occasions within the O-C and O-F plots (31 January and 3 May 1995). The NO fluxes were measured in the field from dynamic chambers (Keller and Reiners, 1994) at each existing chamber anchor using a 20 cm tall Teflon-lined PVC (6 l volume) chamber and a chemiluminesent NO/NO$_2$ analyzer. Methane and N$_2$O fluxes were measured from the same anchors 15–60 min following NO measurements. We measured NO and N$_2$O fluxes from the AO site one week following the quarterly fertilization (31 January 1995).

*Ancillary measurements* On each field sampling day, soils were collected from three plots within each treatment at depths of 0–10, 10–20 and 20–30 cm. Soils were returned to the laboratory and individually analyzed for gravimetric soil water content. Soil temperature at 5 cm was measured at each sampling time at each plot using a hand held digital thermometer. The mean of initial and final air temperature was used in gas flux calculations.

Daily minimum and maximum temperatures and precipitation data were collected at each site. The grass within plots at each location was harvested from the plots monthly by rotary mower.

Soil pH was measured using aqueous saturated paste (McLean, 1982). Soil bulk density was measured by the core method (Blake and Hartge, 1982) at a time when soil moisture conditions (about 50% WFPS) permitted limited compaction.

### Laboratory CH$_4$ uptake induction study

The procedure for a CH$_4$ uptake induction study was adapted from Bender and Conrad (1995). Soil samples (0–15 cm) were collected from each replicate plot of the following treatments O-C, AO-C, AO-L, AO-F and AO-F-L. The soils from each treatment were combined and stored field moist (about 20% gravimetric water content) at 4°C in a refrigerator for less than 1 month until experiments were begun.

*Induction with 10% (v/v) CH$_4$* The day that incubations were initiated, soils were passed sieved (4 mm) to remove large roots and plant residue. For each treatment, 400 g of soil on a dry weight basis, was poured into each of four 1.8-l canning jars. Each jar was sealed with a rubber gasketed lid that was modified to contain a gas sampling septum. Then 120 ml of CH$_4$ was added to two of the four jars from each treatment to provide an air volume of about 10% (v/v) CH$_4$. The jars were boxed and kept outside the laboratory in the shade (temperature range of 24–32°C) for a total of 3 weeks. Weekly the jars were opened to aerate for a few minutes, resealed, and a fresh 120 ml of CH$_4$ was injected into the jar headspace allocated for CH$_4$ induction. After induction and conditioning, soils from the two jars treated with CH$_4$ and two jars incubated in air, for each treatment, were mixed, spread in a < 1-cm layer for 2 h in the laboratory to aerate the soils and remove the excess CH$_4$. The conditioned soils were either used immediately in incubation studies or placed in plastic bags and stored in the refrigerator at 4°C.

*Effect of induction on soil consumption of atmospheric CH$_4$* For each treatment four replicate 100 g (dry weight basis) of soil were weighed into 980-ml canning jars. Each jar was sealed with a rubber gasketed lid that was fitted with a gas sampling septum. Soils from each treatment that had not been conditioned with the high concentrations of CH$_4$ were also incubated. Jar headspace, 15 ml, was sampled by syringe after soils had been kept for 0, 1, 2, 5 and 6 d, in the dark in the shade outside the laboratory (temperature range of about 25–35°C). After each sampling 15 ml of air was injected into each jar to maintain atmospheric pressure inside the jar. At the end of the study the gravimetric moisture content of the soils was 23 ± 3%.

*Statistical analyses*

Within each site, fluxes were measured at six fixed locations at each sampling time. Over the period for which differences were tested, the mean flux rate at each measurement location was calculated, and paired $t$-test or analysis of variance GLC procedures ANOVA performed (SAS, 1991). Distributions were checked for normality using the Shapiro–Wilk statistic. If the normality assumption was not met, then treatments were separated using the Wilcoxon test, otherwise the LSD mean procedure was used to test for N fertilizer, pH or tillage effects. Sites were considered to be significantly different if $P = 0.05$ or less.

### RESULTS

*Experiment 1: Tillage, soil acidification and cropping*

Plowing of the Oxisol had little effect on seasonal or $N_2O$ emission (Table 1). During the first week after tillage, $N_2O$ emissions were about 10-fold higher than the control, but fluxes returned to control rates after that time. Averaged over the entire sampling period (5 October 1993–8 March 1994) mean flux rates in O-C and O-P ($P > 0.1$) and in AO-C and AO-P ($P > 0.1$) were not different (Table 1). Soil acidification also did not appreciably change $N_2O$ emissions. Emissions from the O-C and AO-C as well as O-P and AO-P plots were not significantly different over this period. Nitrous oxide emissions from the sorghum field were almost double those from O-F and 3.5 times those from the O-C site (Wilcoxon test, $P < 0.01$) (Table 1). Fertilization of the Oxisol increased $N_2O$ emissions ($P = 0.02$) as noted for a 28-month observation period in the Oxisol plots (Mosier and Delgado, 1997).

Measurements were continued beyond March 1994 in only the O-C and AO-C sites. When observed over an entire year (2 November 1993 to 25 October 1994) differences between O-C and AO-C mean $N_2O$ emission rates appear. Over the 1-yr period (Fig. 1(a)) $N_2O$ emissions were higher (Wilcoxon text, $P < 0.05$) in the O-C plots, averaging 15.7 compared to 9.6 µg N m$^{-2}$ h$^{-1}$ from

AO-C plots. Within these sites soil water content, mineral N content (data not shown) and temperature did not differ at individual measurement times.

Although plowing the O and AO sites had little effect upon seasonal CH₄ uptake, fertilization of the Oxisol decreased CH₄ uptake ($t$-test, $P = 0.03$). In the sorghum field that had been cropped annually for many years, CH₄ uptake rates were about one-seventh of that in the O-C plots.

Acidification of the Oxisol (AO-C) significantly decreased CH₄ consumption ($t$-test, $P < 0.01$) compared to the O-C site over the 5-month observation period (Table 1). Plowing the acidified Oxisol did not further alter CH₄ uptake rates (Table 1). These lower CH₄ uptake rates in the AO-C and AO-P plots were surprising since pH appeared to have little effect on CH₄ uptake in other ecosystems (Knowles, 1993; Sitaula *et al.*, 1995). Over the year CH₄ uptake rates were significantly lower (Wilcoxon test, $P < 0.05$) in the AO-C than in the O-C sites, averaging 1.2 and 7.5 µg C m$^{-2}$ h$^{-1}$, respectively, over this period (Fig. 1(b)).

*Experiment 2: Effect of liming on CH₄ and $N_2O$ emissions, initial study*

Because of the large differences in CH₄ uptake rates between the O-C and AO-C soils, we conducted an initial liming study. The data discussed in this section were collected from 5 July 1994 to 25 October 1994. During this period CH₄ uptake remained significantly higher in the O-C than the AO-C sites (ANOVA/LSD, $P < 0.05$) (Table 2). Liming resulted in a small but not statistically significant ($P > 0.1$) increase in CH₄ uptake (Table 2). Liming to raise soil pH (0–10 cm) from near 4 to about 6 significantly raised $N_2O$ emissions to the O-C site level. This was the expected response of $N_2O$ emissions to liming (Firestone, 1982), but we expected a larger increase in CH₄ uptake rates.

*Experiment 3: Effect of liming and fertilization on CH₄ and $N_2O$*

To document more clearly the effect of liming and N fertilization on CH₄ and $N_2O$ fluxes in the acidified Oxisol, we conducted an additional 10-month field study within the AO and O sites from 31 October 1994 to 22 August 1995 (Fig. 2). Liming significantly increased ($P < 0.01$) soil pH from less than 4.5 in the surface 0–10 cm of plots AO-C and AO-F to 5.9 and 6.0 in plots AO-F-L and AO-L (Table 3). The pH of the 10–20 cm layers were lower, 5.2 and 5.3 in these plots but were significantly higher than the pH 4.3 and 4.1 in the AO-C and AO-F plots.

Methane uptake rates did not differ (ANOVA, $P > 0.2$) among AO treatments, averaging 0.8, 1.4, 0.3 and 0.8 µg C m$^{-2}$ h$^{-1}$ in AO-C, AO-F, AO-L and AO-F-L, respectively (Table 4). These consumption rates were much lower than either the O-

Table 1. Effects of plowing, fertilization and cropping on CH₄ uptake and $N_2O$ emissions from Oxisol and acidified Oxisol soils from 5 October 1993 to 8 March 1994

| Site/treatment | CH₄ uptake µg C m$^{-2}$ h$^{-1}$ | $N_2O$ emission µg N m$^{-2}$ h$^{-1}$ |
|---|---|---|
| Oxisol-control (O-C) | 7.9 a† | 16.3 c |
| Oxisol-plowed (O-P) | 8.1 a | 11.2 c |
| Oxisol-fertilized (O-F) | 5.2 b | 34 b |
| Acidified Oxisol-control (AO-C) | 2.4 c | 12.2 c |
| Acidified Oxisol-plowed (AO-P) | 2.0 c | 18.0 c |
| Oxisol-sorghum field (Sorghum) | 1.2 c | 57 a |

†Numbers within each column followed by different letters are significantly different (Wilcoxon test, $P < 0.05$).
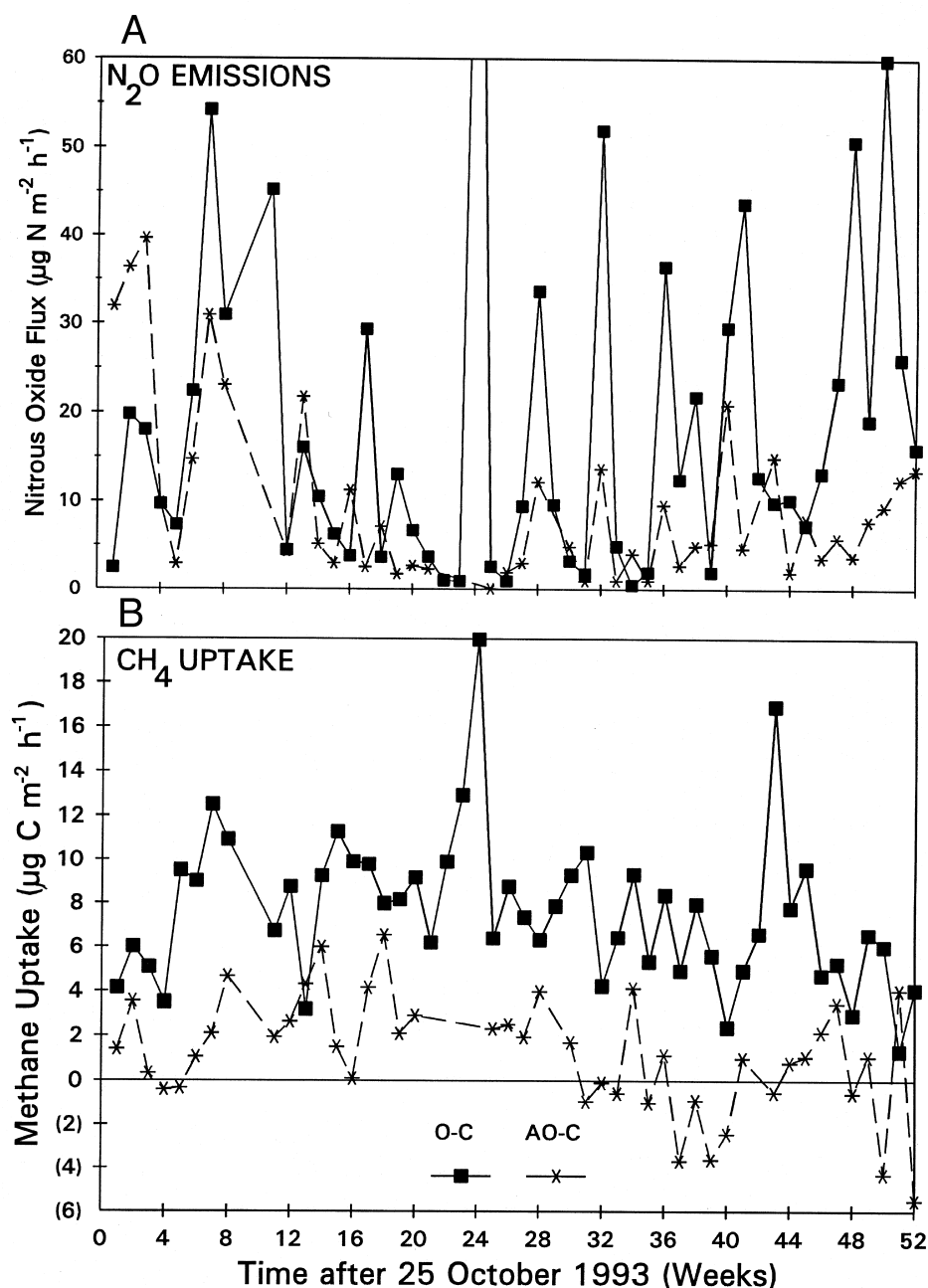
Fig. 1. (a) Weekly treatment mean $N_2O$ emissions from the Oxisol control (O-C) and acidified Oxisol control (AO-C) plots during the period from October 1993 to November 1994. (b) Weekly mean $CH_4$ uptake rates from O-C and AO-C plots during this period

Table 2. Mean $CH_4$ uptake and $N_2O$ emissions from an acidified Oxisol control (AO-C) and limed (AO-L) plots and Oxisol control (O-C) plots over the period from 5 July 1994 to 25 October 1994

| Site/treatment | $CH_4$ uptake $\mu g\ C\ m^{-2}\ h^{-1}$ | $N_2O$ emission $\mu g\ N\ m^{-2}\ h^{-1}$ |
|---|---|---|
| O-C | 6.2 a[†] | 25 a |
| AO-C | −0.4 b | 7.5 b |
| AO-L | 0.8 b | 21 a |

[†]Numbers within each column followed by different letters are significantly different (ANOVA/LSD, $P < 0.05$).

F or O-C rates which averaged 2.8 and 4.8 $\mu g$ $C\ m^{-2}\ h^{-1}$. The lack of $CH_4$ uptake response to liming or fertilization suggests that acidification had greatly affected the community of soil methanotrophs that utilize atmospheric $CH_4$.

Nitrous oxide emission rates were responsive to treatments in the AO site. Liming resulted in small increases in $N_2O$ flux while fertilization alone resulted in significantly smaller increases in $N_2O$.
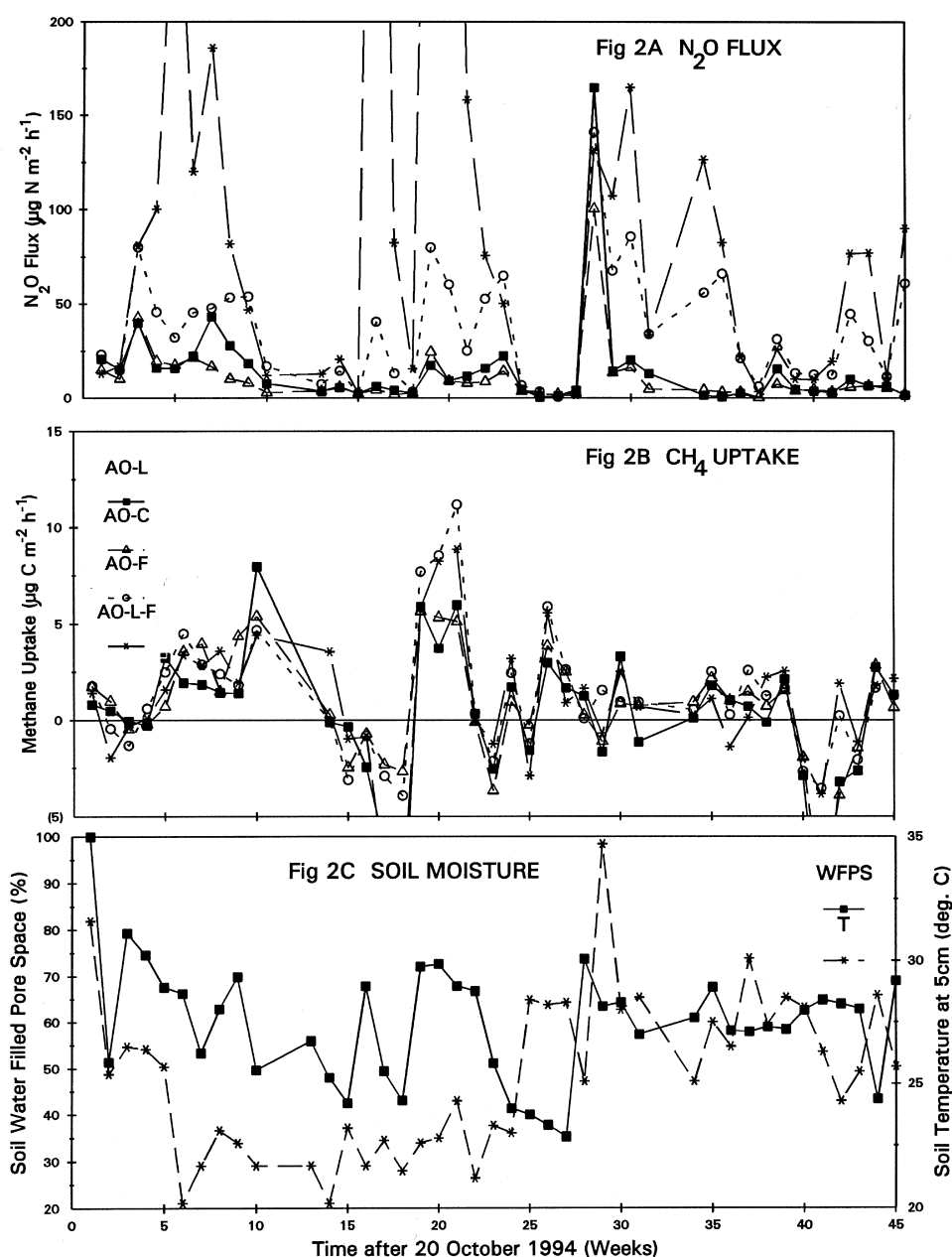
Fig. 2. (a) Weekly mean $N_2O$ emissions from AO-C, AO-L, AO-F and AO-L-F plots during the period from 20 October 1994 to 22 August 1995; (b) weekly mean $CH_4$ uptake in these plots during the period; (c) weekly soil WFPS and 5 cm soil temperature from these plots, during the period

Table 3. Effects of liming on the pH of soils within the acidified Oxisol (AO) site. The data presented are the mean of four separate measurements made from December, 1994 to September, 1995 within each replicate plot of each treatment

| | Soil depth | |
|---|---|---|
| | 0–10 cm | 10–20 cm |
| Treatment | pH | |
| AO-C | 4.4 a[†] | 4.3 a |
| AO-L | 6.0 b | 5.3 b |
| AO-F | 4.0 c | 4.1 a |
| AO-F-L | 5.9 b | 5.2 b |

[†]Numbers in each column followed by different letters are significantly different (ANOVA/LSD, $P < 0.5$).

Both liming and fertilization increased emissions to slightly above those observed from the O-F site (Table 4).

*Experiment 4: Effect of nitrite fertilization and of nitrification inhibitors and ammonium fertilization on NO and $N_2O$ emissions*

A measurement of NO and $N_2O$ emissions, a week after the quarterly fertilization, demonstrated that emissions of both gases were small from the unfertilized soil. This suggests that the availability of mineral N (typically $< 5$ mg kg$^{-1}$) for both limed

Table 4. Effect of liming and fertilization on mean treatment $CH_4$ uptake and $N_2O$ emissions from 31 October 1994 to 22 August 1995

| Site/treatment | $CH_4$ uptake μg C m$^{-2}$ h$^{-1}$ | $N_2O$ emission μg N m$^{-2}$ h$^{-1}$ |
|---|---|---|
| Oxisol-control (O-C) | 4.8 a[†] | 12.8 c |
| Oxisol-fertilized (O-F) | 2.8 b | 85 a |
| Acidified Oxisol-control (AO-C) | 0.8 c | 10.8 c |
| Acidified Oxisol-fertilized (AO-F) | 1.4 c | 36 b |
| Acidified Oxisol-limed (AO-L) | 0.3 c | 14.6 c |
| Acidified Oxisol-fertilized-limed (AO-F-L) | 0.8 c | 102 a |

[†]Numbers in each column followed by different letters are significantly different (ANOVA/LSD, $P < 0.05$).

Table 5. NO and $N_2O$ fluxes measured 1 week following the quarterly fertilization in the AO site

| | NO | | $N_2O$ | |
|---|---|---|---|---|
| | Mean | S.E.[†] | Mean | S.E. |
| Treatment | | μg N m$^{-2}$ h$^{-1}$ | | |
| AO-C | 20 | 2 | 8 | 2 |
| AO-F-L | 2290 | 1192 | 630 | 260 |
| AO-L | 13 | 4 | 11 | 3 |
| AO-F | 240 | 65 | 63 | 9 |

[†]S.E. = standard error of the mean, $n = 6$.

and unlimed plots limited emissions (Table 5). In the fertilized plots both NO and $N_2O$ emissions were large, with large plot to plot variability. Emissions were about 10-fold higher from the limed plots.

Addition of nitrite greatly increased NO and $N_2O$ emissions, very quickly (Table 6). The increase in NO emissions was almost 15 times higher from the unlimed, pH 4.3, soil compared to the limed, pH 6 (Table 4).

NO was emitted from ammonium fertilized soils in about the same amounts as from limed and unlimed soils at both 2 and 24 h after fertilization (Table 7). $N_2O$ emissions were lower from the unlimed soils where NO-to-$N_2O$ ratios were 8.5 and 31, in limed and unlimed fertilized plots, respectively. Both nitrification inhibitors significantly decreased NO and $N_2O$ emissions from the limed soils but only $C_2H_2$ decreased NO and $N_2O$ emis-

sions from the unlimed soil (Table 7). Acetylene is well known to inhibit nitrification (Walter *et al.*, 1979) in a variety of field conditions (Mosier *et al.*, 1994). DCD was inactivated in the acid soil, possibly through a mechanism where protonated amino groups of the DCD were bound to soil humic material carboxyl groups by ionic bonding (Jacinthe and Pichtel, 1992).

### Laboratory $CH_4$ uptake induction study

Since liming did little to increase soil $CH_4$ uptake rates in the AO soils we attempted to induce an increase in $CH_4$ uptake by these soils. We incubated surface 0–15 cm depth soils from the O-C site and the AO site treatments with 10% $CH_4$ atmosphere in air for 3 weeks. We then measured $CH_4$ uptake rates of these conditioned soils in a normal atmosphere.

There was limited response of $CH_4$ uptake in the AO soils to induction by conditioning the soils with 10% $CH_4$ in air (Table 8). In the AO-F-L soil, $CH_4$ consumption increased following the induction conditioning from an undetectable amount to near the preinduction value for the O-C soil. The response in this soil apparently required both increased pH and N fertilization as observed by Bender and Conrad (1995). The O-C soil $CH_4$ uptake rates were increased almost 4-fold by induction.

### DISCUSSION

#### Nitrous oxide

Nitrous oxide emissions from the pH 4 acidified Oxisol (site AO-C) were less than from the pH 6 Oxisol (site O-C) soils (Fig. 1(a); Table 2) during the year from October 1993 to November 1994. In the following year (1994–1995 Fig. 2(a); Table 4), however, no difference in $N_2O$ emissions was observed between pH 6 and pH 4 soils. Plowing the grasslands did not change the mean $N_2O$ emission rates during the measurement period (Table 1). It was surprising that $N_2O$ emission rates were not

Table 6. Effect on NO and $N_2O$ emissions of adding 6 mm of water with or without $NaNO_2$ (0.75 kg N ha$^{-1}$) to microplots within the AO-C and AO-L treatments in the acidified Oxisol (AO) site

| Time after amendment (h) | Amendment | | | | | | | | Soil water filled pore space (WFPS) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $H_2O$ | | | | $H_2O + NaNO_2$ | | | | | |
| | NO | S.E.[†] | $N_2O$ | S.E. | NO | S.E. | $N_2O$ | S.E. | Mean | S.E. |
| | | | | μg N m$^{-2}$ h$^{-1}$ | | | | | % | |
| Limed (AO-L) | | | | | | | | | | |
| 0.5 | 56 | 23[†] | 32 | 16 | 1120 | 560 | 480 | 235 | 44.1 | 2 |
| 4 | 130 | 60 | 41 | 15 | 1020 | 475 | 280 | 245 | 50.8 | 0.9 |
| Unlimed (AO-C) | | | | | | | | | | |
| 0.5 | 93 | 10 | 24 | 7 | 16 200 | 9175 | 1500 | 965 | 44.7 | 1.6 |
| 4 | 200 | 15 | 20 | 9 | 3030 | 1460 | 1190 | 450 | 51.7 | 3.7 |

[†]Mean treatment flux rates followed by standard error of the mean, $n = 4$.

Table 7. Effect of liming, ammonium fertilization and nitrification inhibitors on NO and N$_2$O emissions from microplots within AO-C and AO-L treatments in the acidified Oxisol (AO) site

| | 2 h | | | | 24 h | | | | 8 d | |
| | NO | | N$_2$O | | NO | | N$_2$O | | N$_2$O | |
| Treatment | Mean | S.E.[†] | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. |
| | | | | | μg N m$^{-2}$ h$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Limed (AO-L)** | | | | | | | | | | |
| NH$_4$ | 400 | 130 | 47 | 6 | 250 | 35 | 30 | 8 | 870 | 140 |
| NH$_4$ + DCD[‡] | 84 | 42 | 31 | 15 | 59 | 16 | 13 | 3 | 340 | 100 |
| NH$_4$ + ACT[‡] | 62 | 21 | 27 | 6 | 11 | 3 | 15 | 5 | 530 | 35 |
| **Unlimed (AO-C)** | | | | | | | | | | |
| NH$_4$ | 400 | 157 | 13 | 1 | 240 | 30 | 11 | 1 | 340 | 135 |
| NH$_4$ + DCD | 320 | 80 | 48 | 15 | 270 | 25 | 10 | 2 | 320 | 55 |
| NH$_4$ + ACT | 130 | 60 | 5 | 2 | 67 | 23 | 4 | 1 | 200 | 26 |

[†]S.E. = standard error of the mean, $n = 4$.
[‡]DCD = the nitrification inhibitor dicyandiamide; ACT = acetylene.

always significantly lower (ANOVA, $P > 0.1$) from the AO-C and AO-P soils than from the O-C and O-P plots. Since the pH was about 6 in the O-C and O-P soils we expected to observe greater N$_2$O emission rates as higher total N mineralization and nitrification rates were expected (Alexander, 1965; Broadbent and Clark, 1965; Schmidt, 1982). Although we did not measure these rates, possibly total N turnover rates were lower in the AO sites and N$_2$O + N$_2$ from denitrification was limited mostly to N$_2$O (Firestone, 1982). In experiment 2 (Table 2) where data were taken from an adjacent location during a different period, N$_2$O emissions were lower from the AO-C plots. The reason for this mixture of results is unclear, but further demonstrates the need for long-term observations.

Cropping and fertilization of sorghum resulted in about a 5-fold increase in N$_2$O emissions relative to the unfertilized, plowed grassland (site O-P) and about a doubling of emissions compared to the fertilized grassland (site O-F) (Table 1). We expect that soil disturbance due to weeding operations and an occasional water supplement during the growing season contributed to the additional N$_2$O emission from the sorghum field.

Liming the acidified Oxisol tended to increase N$_2$O emissions from the unfertilized plots (AO-L) to the level of the unamended Oxisol (O-C) (Tables 1, 2 and 4). The liming response was greater during the initial, short-term study (Table 2) than the longer-term measurement period of the second liming study (Table 4). The general trends, however,

were the same for both periods. Fertilization increased N$_2$O emissions about 3-fold from the limed plots (AO-F-L) compared to unlimed plots (AO-F) (Table 4).

Short-term measurement of NO and N$_2$O emissions indicate that in the AO site both NO and N$_2$O emissions are likely regulated by ammonium availability and nitrification rate (Tables 5 and 7). Adding nitrite to either limed or unlimed plots increased NO and N$_2$O emissions very quickly (Table 2), and in a much higher NO-to-N$_2$O ratio, 2.3 compared to 10.7, in the unlimed plots. Although microbial production of NO cannot be precluded (Davidson, 1992; Yamulki et al., 1997), it appears that NO was produced through the chemical decomposition of nitrite, which in turn was a product of biological nitrification. This indicates that the gases are probably produced mainly by chemodenitrification in the acid soils (Broadbent and Clark, 1965; Parsons and Keller, 1995; Yamulki et al., 1997). When nitrite is formed from ammonium oxidation, and it is in soil solution, it is likely quickly converted to NO chemically at low pH (Yamulki et al., 1997).

The nitrification inhibitor study also suggests that NO and N$_2$O emissions are first regulated by ammonium oxidation. Addition of 7.5 g N m$^{-2}$ of ammonium sulfate, if mixed uniformly throughout the top 5 cm of soil, would provide 110 μg N g$^{-1}$ of soil. This is likely sufficient ammonium to saturate nitrifier demand (Schmidt, 1982). As a result, liming did not greatly increase NO and N$_2$O emissions, 4

Table 8. Cumulative amount of CH$_4$ consumed during a 6-d incubation following a 3-week induction of the soils with 10% (v/v) CH$_4$ in air

| | Sites/treatments from which soils were collected | | | | |
| | O-C | AO-C | AO-F | AO-L | AO-F-L |
| | | | ng CH$_4$ consumed g$^{-1}$ soil | | |
|---|---|---|---|---|---|
| No induction | 2.1 a | 1.2 a | ND[†] a | ND a | ND a |
| After induction | 8.2b | ND a | ND a | ND a | 3.2 b |

[†]ND indicates no detectable CH$_4$ consumption and numbers in columns followed by different letters are significantly different ($t$-test, $P < 0.05$).

to 24 h after N additions (Table 7). Addition of nitrification inhibitors, DCD or acetylene, both inhibited NO and $N_2O$ emissions from limed soils. In unlimed soils DCD did not inhibit NO or $N_2O$ emissions, indicating either a different microbial process for NO and $N_2O$ production in the unlimed soil or, more likely, that DCD was inactivated by binding to humic compounds under acidic conditions (Jacinthe and Pichtel, 1992).

These data further indicate that the reduction of nitrite proceeds mainly through a chemical process in acid soils following the oxidation of ammonium. When ammonium concentrations were high, and not rate limiting, nitrification rates were not decreased by acidic soil conditions. A week after ammonium addition, when soil ammonium content should have been much lower following a week of microbial and plant uptake, $N_2O$ emissions were significantly higher (*t*-test, $P < 0.05$) from the limed soil (Table 7). Both nitrification inhibitors appeared to decrease $N_2O$ emissions from the limed soil but only acetylene continued to decrease $N_2O$ emissions from the unlimed soil. A complete inhibition of nitrification by acetylene would not be expected, however, 8 d after acetylene was added to the chamber headspace (Klemedtsson and Mosier, 1994). These data further suggest that nitrification rates were lower in the unlimed soil and that NO was produced in large part through chemical processes and $N_2O$ through microbial processes (Broadbent and Clark, 1965; Yamulki *et al.*, 1997). Soil water contents ranged between 15 and 20% (42–56% WFPS) during these studies. At this moisture content range nitrification should have been active and denitrification limited by oxygen availability (Linn and Doran, 1984). Although NO formation is likely due to the chemical decomposition of nitrite at low pH, nitrite is produced and consumed in large part by biological processes, as indicated in the short-term studies. This suggests that relative to the transformation rate, the pool size of nitrite is small in unfertilized systems and that the biological nitrification controls dominate the controls on NO production and emission as they dominate the production and emission of $N_2O$.

The NO-to-$N_2O$ emission ratio data also suggest that nitrification rates were controlling emissions and that $N_2O$ emissions were limited by nitrification rather than by the $N_2$-to-$N_2O$ ratio of denitrification. Since the NO-to-$N_2O$ ratios were the same in AO-F-L and AO-F plots, 3.6 and 3.8, this also suggests a nitrification control on $N_2O$ emissions since NO emissions are generally attributed to nitrification (Williams *et al.*, 1992).

*Methane uptake*

Methane uptake was not noticeably affected by plowing in either the O or AO sites, but was decreased in the Oxisol by fertilization. Methane uptake was decreased further in the fertilized, sorghum-cropped soils (Table 1). This relative difference between grassland and cultivated field $CH_4$ uptake rates is similar to that observed for systems in semiarid Colorado (Bronson and Mosier, 1993). Methane uptake rates averaged 4.3 times higher in the fertilized grassland (O-F) than in the sorghum field. This difference could not be attributed to pH since the pH of the O-F site and sorghum were both between 5.5 and 6.0, but may be related to the effect of N turnover on methanotrophs in the cultivated soil. Acidification of the Oxisol considerably decreased $CH_4$ uptake rates to about one-fourth of those observed in the control Oxisol. Liming the soils back to pH 5.5 to 6 did not rejuvenate the $CH_4$ uptake, providing only very small, but not statistically significant increases ($P > 0.2$, Tables 2 and 4). Hütsch *et al.* (1994) also showed that both lower pH and N additions decreased $CH_4$ emission from grasslands in central England.

Methane oxidation rates that are very low at normal atmospheric concentrations of $CH_4$ can be increased by inducing oxidation with high concentrations of $CH_4$ (Nesbitt and Breitenbeck, 1992; Bender and Conrad, 1995). We conducted a series of $CH_4$ uptake induction studies with AO and O-C site soils and found little positive inductive response in the AO soils (Table 8). Only in limed and fertilized plots did the induction increase $CH_4$ uptake. Possibly both liming and fertilizing with ammonium increased the nitrifier communities which also oxidize $CH_4$. In the O-C soils the induction increased uptake about 4-fold, indicating that the process will function under appropriate conditions. The induced increase in $CH_4$ uptake in the O-C soil followed the pattern suggested by laboratory studies from Nesbit and Breitenbeck (1992) and Bender and Conrad (1995). They indicated that such induction could stimulate consumption of atmospheric $CH_4$ when appropriate methanotrophic communities are present and no physical or chemical limitations are imposed. Bender and Conrad (1995) showed that $CH_4$ consumption with 20% $CH_4$ in our air atmosphere occurred within 16 d. They found that the pH optima for the acid soil in their investigation (in situ pH 4.5) were about 6.3. In this soil they observed slow $CH_4$ uptake even when pH was lowered to 2.3. Soil moisture and temperature optima were both well within the range of those used in our study. They also found that low concentrations of ammonium stimulated both induction and induced $CH_4$–oxidizing activity. In the acidified Oxisol soil either methanotrophic communities were not present because of the acidification process or some unidentified factor was missing or present in toxic amounts.

From these studies it is apparent that land use history, soil pH and ammonium fertilizer inputs all have marked and interacting effects on soil mi-

crobial processes, methanotrophy and nitrification. There are also marked similarities between N$_2$O emission and CH$_4$ consumption responses to fertilizer input and soil acidification between the central England (Hütsch *et al.*, 1994) and western Puerto Rico grasslands. These similarities suggest the potential for general process descriptions which transcend ecosystem boundaries that may be applicable for planning of land use and agricultural practices that will maximize the extent to which aerobic soils can act as sinks for CH$_4$ and the extent of N$_2$O emissions to the atmosphere.

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